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SUBALPINE SOIL ECOLOGY & CAMPGROUND RESTORATION RESEARCH CONDUCTED FOR THE ALDO LEOPOLD WILDERNESS RESEARCH INSTITUTE, 1992-1996: FINAL REPORT

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Subalpine Soil Ecology and Campground Restoration Research Conducted for the Aldo Leopold Wilderness Research Institute, 1992-1996: Final Report

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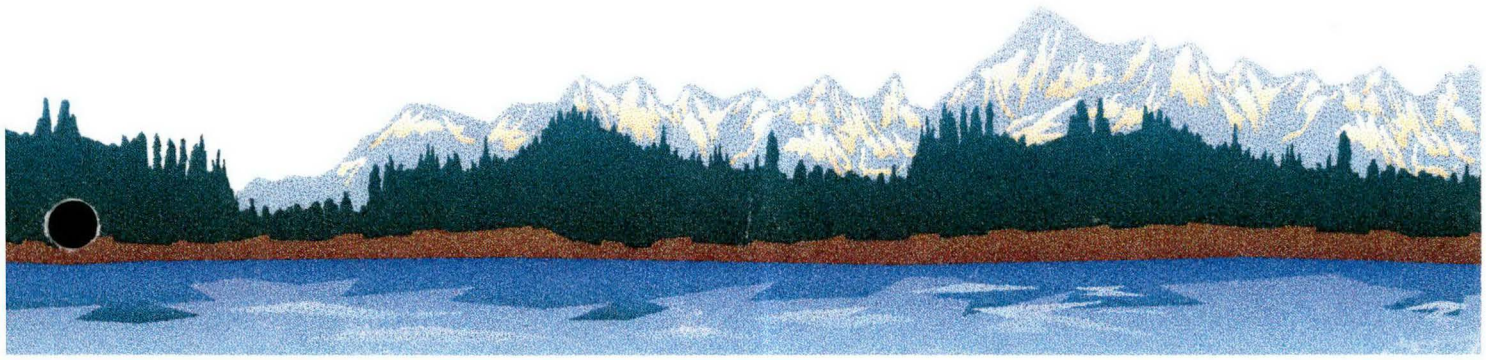

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Contents

Introduction....2

Section 1. Soil foodweb shifts and reduced plant nutrient supply in a degraded subalpine recreation site....4

Section 2. Increased bacterial denitrification in two subalpine recreation campsites....23

Section 3. Pinegrass (*Calamagrostis rubescens*) facilitates vesicular-arbuscular mycorrhizal colonization and phosphorus uptake in subalpine fir (*Abies lasiocarpa*)....25

Section 4. Effects of three *Phialocephala*-like fungal inocula on survival and nutrient status in *Abies lasiocarpa* and *Pinus contorta*....37

Section 5. Summary of seeding trials and *Carex* field plot results....42

Conclusions and Recommendations....44

Introduction

This report details the results of a four-year study conducted for the Leopold Institute in Missoula, Montana. Field work was conducted in Glacier National Park, Montana. Laboratory and greenhouse experiments using native Montana plant species were conducted at Oregon State University, Corvallis Oregon.

The broad subject of the study was the degradation and restoration of impacted subalpine recreation areas. The majority of the research elements (field sampling and experiments) described here focus on soil ecology and nutrient cycling relevant to native plant establishment and survival. Mycorrhizae, nutrient pools and pathways, and soil foodweb structure and function are addressed. **Section 1** is presented in manuscript format and is in preparation for journal submission. It describes the results of baseline sampling at Cobalt Lake campground, conducted to improve our understanding of how the organisms that confer soil fertility are impacted by years of recreationist trampling and camping. **Section 2** summarizes the results of an additional component of baseline sampling conducted at Cobalt Lake and Red Eagle campgrounds. The focus of the soil analysis was the potential for the alteration of normal nitrogen pathways resulting from a shift in the composition of the bacterial community. **Section 3**, presented in manuscript format, describes the results of a laboratory experiment testing the belowground benefits of growing plants of very different species (and life forms) in close proximity. The presence of pinegrass facilitated vesicular-arbuscular mycorrhizal colonization of subalpine fir, and resulted in a 10-fold increase in phosphorus uptake. **Section 4** summarizes the results of greenhouse experiments conducted to test for the possible benefits of a little-known class of fungal endophyte that is extremely common on the roots of subalpine and alpine plants. Though difficult taxonomically, the fungi are relatively easy to isolate and propagate, and may offer some promise as a restoration tool. A bibliography of relevant journal articles is included. **Section 5** summarizes the results of an ongoing field experiment at Cobalt Lake using a common subalpine sedge planted in degraded areas. It tests the effects of 1) the fungal inoculum described in Section 4, 2) perlite addition to the

root zone, and 3) an untreated control. It also describes efforts to revegetate experimental plots by directly seeding beargrass and sedge (both requiring a long period of cold moist stratification) in October, and then protected from over-winter seed predation. Though no seedlings germinated in the plots, the over-winter protection was markedly successful. Seed loss occurred after the protective screens were removed in the spring.

This project was funded by the Leopold Institute. We thank Dave Cole of the Institute for his active participation and for his patience. Support from Glacier National Park managers and what was then the Glacier Field Unit of the National Biological Service made the field work possible. The OSU Department of Forestry provided greenhouse and laboratory space and equipment. Jane Smith of the USFS Forestry Sciences Laboratory in Corvallis helped develop and conduct the study described in Section 3. The soil chemical analyses were overseen by Carol Glassman of the Department of Forest Science. Soil biological analyses were conducted in the laboratory of Elaine Ingham, Department of Botany. Denitrification analyses were conducted in the laboratory of Bob Griffiths, Department of Forest Science. Joyce Lapp at the Glacier Park native plant nursery provided plants, seeds and technical assistance. The Native Plant Nursery crew and the staff at Two Medicine Ranger District generated considerable interest and enthusiasm, lending much to the enjoyment of the project.

Soil foodweb shifts and reduced plant nutrient supply in a degraded subalpine recreation site

Abstract

Soils from a degraded wilderness recreation site at Cobalt Lake in Glacier National Park were analyzed to determine whether nutrient loss or limitation might be a factor in poor site recovery, and whether limitation could be related to changes in the activity of soil organisms. Surface soils were sampled in a severely compacted, devegetated campsite, then compared to samples from adjacent undisturbed and moderately damaged areas. Whole soil samples were extracted for available P and combusted for total C and N. Ion exchange resin bags embedded at 30 sampling points for 2 time intervals were assayed for NO_3, NH_4 and P. A greenhouse bioassay for foliar N and P was conducted using a native grass grown in soils collected at the site. Organisms representing two foodweb trophic levels were assessed microscopically. Active bacterial and fungal biomass was quantified, together representing the first-level decomposers. Nematode and protozoan individuals were counted tallied, together representing second-level consumers. Spores of vesicular-arbuscular mycorrhizal fungi were enumerated in the same samples, and roots of the bioassay grasses were examined for mycorrhizal colonization.

Measures of total N, extractable P and exchange resin NO_3 and NH_4 did not support the hypothesis that N and P availability was limited in the degraded soil. Resin $N0_3$, NH_4 and whole-soil extractable P were, in fact, significantly higher in those soils. Conversely, P extracted from resin bags buried in the degraded site for the 5-week, late summer interval was significantly lower than from the undisturbed site. There were no differences in resin P over the 10-month interval. The resins may be the more realistic indicator of plant-available P in degraded soils, at least for comparative purposes. Results of the greenhouse bioassay support this suggestion because foliar P levels were significantly higher in plants grown in soil from the undisturbed area. Exchange resins were not an accurate indicator of N availability, however, because bioassay plants also took up more N from the undisturbed soils. No fungal colonization of roots was detected in the greenhouse plants, so increased N and P uptake cannot be attributed to mycorrhizae. There was no difference in vesicular-arbuscular spore counts among the three soil types. Disturbance-related shifts were detected, however, in decomposer and consumer trophic levels of the soil foodweb. Active bacterial and fungal biomass was lowest in the area of intermediate disturbance. Total fungal biomass was lowest in the degraded soil. Nematode individuals were 10 times higher in undisturbed soil than degraded soil. Protozoa were highest in the area of intermediate disturbance. Since mycorrhizae were not a factor in nutrient uptake in the greenhouse bioassay, yet plants in undisturbed soils had higher foliar nutrients, it appears the diminished role of nematodes functioning as mineralizers may have reduced nutrient supply to bioassay plant roots. Determining whether human impact leads to disruption in the structure and function of the second trophic level, via soil compaction, could prove pivotal in understanding and ameliorating poor site recovery.

Introduction

Remote montane preserves degraded by excessive or inappropriate recreation activities are extremely difficult to restore, especially if heavy use continues (see Cole and Hall 1992; Cole and Trull 1992; Rochefort and Gibbons 1992). In addition to severe climate, access is poor and choices for revegetation species are limited. We know little about the ecology of soil fertility and degradation in these native ecosystems because they are so remote and, until recently, have been infrequently utilized for commercial timber harvest.

Subalpine forests are characterized by cold temperatures, heavy snowpacks, slow growth rates and limited soil development (Vogt et. al.1989; Baron 1992). Because the forest floor microbial activity that contributes to soil fertility through N mineralization is temperature limited, the organic horizons become important sinks of total ecosystem nitrogen, and the role of distinctive mycorrhizae in nutrient uptake is increased (Read and Haselwandter 1981; Vogt et. al. 1989; Read 1991).

High elevation restoration research typically focuses on the decline or recovery of plant species and communities (e.g. Chambers et. al. 1984; Densmore and Holmes 1987; Chambers et. al. 1990; but see Allen et. al. 1987). Where failure to establish plant cover in native ecosystems is a persistent problem, closer examination of plant-soil interactions are necessary.

Perry et. al. (1989) characterized the relationship between vegetation and soil as a dynamic, self-maintaining system with myriad internal linkages and positive feedbacks operating within environmental constraints, and compared it to the analogous boy who pulls himself up by his own bootstraps (see also Perry and Amaranthus 1990). Within a bootstrapping framework, internal linkages and interactions mediate system resilience and productivity. Seemingly redundant pools and pathways confer stability. Biological components of soil, including plant roots, mycorrhizae and rhizosphere organisms are major contributors to internal nutrient linkages and pathways. For example, plant litter production and the active release of carbon compounds from roots (Paul and Clark 1989) provides energy to microbial decomposers (bacteria and fungi) that in turn are food for larger grazers

and consumers (nematodes, protozoa and microarthropods). Because the concentration of N in decomposer tissue is high, their consumers excrete excess N, typically in forms available for plant uptake and growth (Anderson et. al. 1981), allowing for continued photosynthesis and litter production. For the techniques of restoration ecology to evolve beyond revegetation, an understanding of how plant-soil linkages and feedbacks unravel during degradation is critical.

Because of the strong influence of soil biota on major plant nutrients (Paul and Clark 1989; Baere et. al. 1995), functional characterizations or models of soil organisms have been developed and tested. (Coleman 1985; Moore et. al. 1988; Walter et. al. 1991). As a basis for a foodweb conceptual model, Ingham et. al. (1985) summarized system responses to the consumption of decomposers by consumers : increased N and P mineralization, increased $C0_2$ evolution, increased plant N uptake and growth, and increased or decreased bacterial populations. In a grassland study of seasonal relationships between decomposers and consumers, Ingham et. al. (1986) provided evidence that consumer populations tracked their resource base through time, and further, that increased N mineralization occurred concomitant with higher consumer numbers. During a late-summer period when bacterial numbers increased fourfold, N was immobilized. Figure 1 (derived from Ingham et. al. 1986) shows simplified component groups of the first two soil foodweb trophic levels.

A broad objective of the study described here was to examine the importance of soil biological activity to the maintenance of a dynamic plant-soil nutrient cycle (presented as a hypothesis by Anderson et. al. 1981), within the conceptual framework of a bootstrapping system. Soil C, N, P and plant tissue N and P were analyzed in relation to the activity of two major foodweb functional groups (N-immobilizing decomposers and N-mineralizing consumers) along a gradient of site degradation.

A final objective was to determine whether the measurement of major component groups in one or two foodweb trophic positions could contribute a functional characterization of site degradation useful to restorationists, based on the findings of Curry and Good (1992), Wardle et. al. (1993) and Ingham and Thies (1995) who suggest that the condition of the detrital foodweb can be a useful indicator of disturbance regimes.

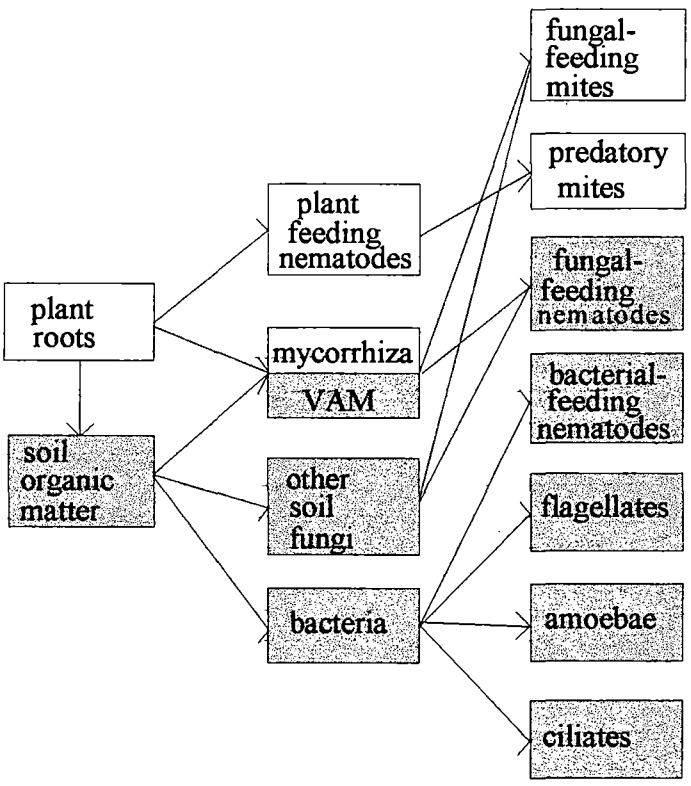

Figure 1. Simplified component groups of the first two soil foodweb trophic levels. Arrows indicate directions of energy and/or nutrient flows between components. Shaded blocks are the points of focus in this study. Modified from Ingham et. al. 1986.

Materials and Methods

Study Site

Cobalt Lake backcountry campground in Glacier National Park is near alpine treeline at 2012m. Elevation. *Abies lasiocarpa* is the only tree species in the area, with patchy groves occurring around the campground. Stunted trees were present in and around the sampling area. Understory vegetation is dominated by *Luzula hitchcockii, Xerophyllum tenax, Vaccinium scoparium,* and various grasses and sedges.

An area of heavy camper use was selected for intensive study and soil samples were collected along a gradient of obvious impact ranging from slight to severe. At the least disturbed end, vegetative cover was intact and consisted mostly of *Abies lasiocarpa, Vaccinium scoparium* and *Xerophyllum tenax*. The adjacent intermediate area had sparse vegetation and a high proportion of bare, compacted soil. The degraded area was a tent campsite completely denuded of vegetation with compacted soil.

Sampling Design

Gridded plots ($3.5m^2$, 0.5m grid) were established in adjacent "undisturbed", intermediate and severely degraded areas. Surface soils were sampled to 15cm at ten randomly selected intersections within each of the three areas, for a total of 30 samples. Samples were not composited. Nylon mesh bags containing 5g wet weight mixed-bed ion exchange resins (Rexyn 300 H-OH, Fisher Scientific) were placed 5cm deep near each sampling hole. The first set was in place for 5 weeks in late August and September, then removed and replaced by a second set which was in place for the subsequent 10 months

Nutrient Analyses

Total C and N were determined by high temperature dry combustion in a Carlo-Erba 1500 Series II Analyzer, following standard manual procedures. Extractable P was assayed using the dilute acid-flouride technique in Olson and Sommers (1982). Soil pH was measured following McClean (1982) in a 1:10 soil/water solution. Resin analysis for P and

N was conducted using 1.5g dry weight resin from each bag extracted in 2N KCL (Hart and Firestone 1989).

Organisms

Active bacteria and fungi were enumerated in diluted soils stained with fluorescein diacetate using epiflorescent microscopy (Ingham 1992; Ingham and Klein 1984; Babiuk and Paul 1970). Most Probable Number enumeration of protozoa followed (Ingham 1992; Darbyshire et. al. 1974). Nematodes were extracted on Baermann funnels (Anderson and Coleman 1977) and counted and identified using DIC microscopy. VAM spores were extracted following Ianson and Allen (1986). Organisms, biomass and spores are reported on a per gram dry soil basis, except nematodes, which are reported on a per 10g dry soil basis.

Greenhouse Bioassay

For the greenhouse bioassay soils were collected from the site in late September, sieved to 1cm and mixed with a standard greenhouse mix of peat, perlite and vermiculite in a 2 to 1 soil/medium ratio. Six-week old seedlings germinated in vermiculite of *Deschampsia caespitosa* were transplanted into the mixture in 150 cc leach tubes. Thirty five leach tubes were planted for each of the three soil types. The plants were watered daily and harvested after 12 weeks. Foliage was oven-dried overnight at 70C and roots were cleared and stained with trypan blue modified from Càsares and Trappe (1993) and Phillips and Hayman (1970). Foliar tissue was analyzed for percent N and P using the micro-Kjeldahl digest described in Bremner and Mulvaney (1982), then processed on a Technicon Autoanalyzer.

Statistical Analyses

Variables were analyzed with one-way analyses of variance followed by Tukey pairwise comparisons using SYSTAT™ Version 5.0 software. Resin data were log-10 transformed to stabilize variance. Due to a laboratory error that reduced nematode analysis to samples sizes of 3,4 and 5 for undisturbed, intermediate and degraded soils respectively,

non-parametric analysis of ranks was employed on all of the organism variables, for consistency. Non-parametric analyses of variance are appropriate where sample sizes are small and equal population variance assumptions are not met (Zar 1984).

RESULTS

Nitrogen

Total soil N at the site was in the range of 0.4% and did not differ significantly ($p \geq .05$) among the three site conditions (Table 1). Mean resin NO_3 in the degraded site for the 5-week and 10-month intervals was 7.6 and 67 times higher, respectively, than resin NO_3 in the undisturbed soils (Table 2 and Figure 2). Both differences were highly significant ($p \leq .001$). Mean resin NH_4 did not differ significantly among the three sites during the short interval. Over the 10-month interval, however, resin NH_4 was 14 times higher in the degraded site than in the undisturbed site ($p \leq .001$).

	Degraded	Deg *vs* Int p value	**Intermed.**	Int *vs* Und p value	**Undisturb.**	Und *vs* Deg p value
%C	**6.93** 1.45	0.849	**6.27** 1.85	0.028	**20.9** 1.97	0.089
%N	**0.457** 0.089	0.403	**0.380** 0.092	0.384	**0.459** 0.188	0.999
P ppm	**41.78** 9.85	0.002	**26.87** 10.7	≤ 0.001	**6.95** 1.53	≤ 0.001
C:N	**15** 0.82	0.036	**16.3** 1.25	≤ 0.001	**20.9** 1.2	≤ 0.001

Table 1. Nutrient means (standard errors listed beneath) and p values for Tukey pairwise comparisons.

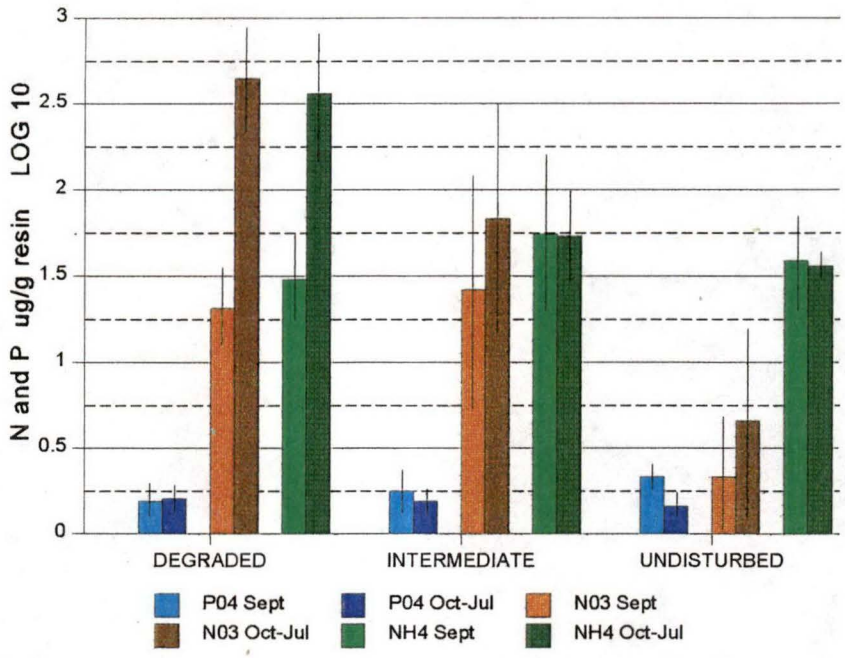

Figure 2. Nutrient extractions from ion exchange resins, log-10 transformed. Error bars are standard error.

	Degraded	Deg *vs* Int p value	**Intermed.**	Int *vs* Und p value	**Undisturb.**	Und *vs* Deg p value
PO4 5wks	**1.59** 0.41	0.264	**1.87** 0.71	0.525	**2.18** 0.39	0.032
PO4 10mos	**1.63** 0.31	0.754	**1.56** 0.26	0.893	**1.47** 0.24	0.459
N03 5wks	**23.7** 13.7	$\leq$0.001	**80.39** 117.1	0.883	**3.03** 3.65	$\leq$0.001
N03 10mos	**543.7** 317.8	$\leq$0.001	**144.5** 148.3	0.011	**8.06** 7.51	$\leq$0.001
NH4 5wks	**36.75** 32.77	0.663	**101.9** 135.5	0.293	**178.6** 50.14	0.786
NH4 5mos	**516.1** 479.9	0.413	**64.86** 48.24	$\leq$0.001	**36.37** 6.44	$\leq$0.001

Table 2. Ion exchange resin results. Untransformed means (standard errors beneath). P values are for Tukey pairwise comparisons made on log-10 transformed data.

Carbon and C:N Ratio

Total soil C ranged from 6 to 21%. It lowest in the disturbed and intermediate sites, 6.3 and 6.9% respectively, but only the difference between intermediate and undisturbed was significant. The constant decline in the ratio of C to N from undisturbed to degraded was highly significant.

Phosphorus

Whole-soil extractable P was 6 times higher in the degraded soils ($p \leq .001$) than in the undisturbed soils. Conversely, resin P was lower in the degraded soils than in the undisturbed ($p \leq .05$), and not significantly different over the long interval.

Organisms

Bacteria, fungi and nematodes all showed significant disturbance-related declines (Table 3 and Figure 3). Protozoans showed a disturbance-related increase. There was no difference in mean active bacterial or fungal biomass between the degraded and undisturbed soils, though active fungal biomass in the degraded soil was less than half that in the undisturbed soil ($p = .116$). Active biomass of both bacteria and fungi was significantly lower in the area of intermediate disturbance, however. The decline in the ratio of active fungi to bacteria from undisturbed to degraded soil was not significant. Total fungi, including both live and dead hyphae, was 3 times higher in the undisturbed soil than in the degraded soil.

Protozoan individuals (mostly flagellates and amoebae), were highest in the intermediate soils, where they were 7 times higher than in the undisturbed soils. Few ciliates were detected in the samples.

Nematode individuals, dominated by bacterial and fungal feeders, were nearly 10 times more abundant in the undisturbed soils than in the degraded soils, and more than twice as abundant in the intermediate soils.

VAM spores were present in all three soils, but counts were highly variable and there were no significant differences in quantity.

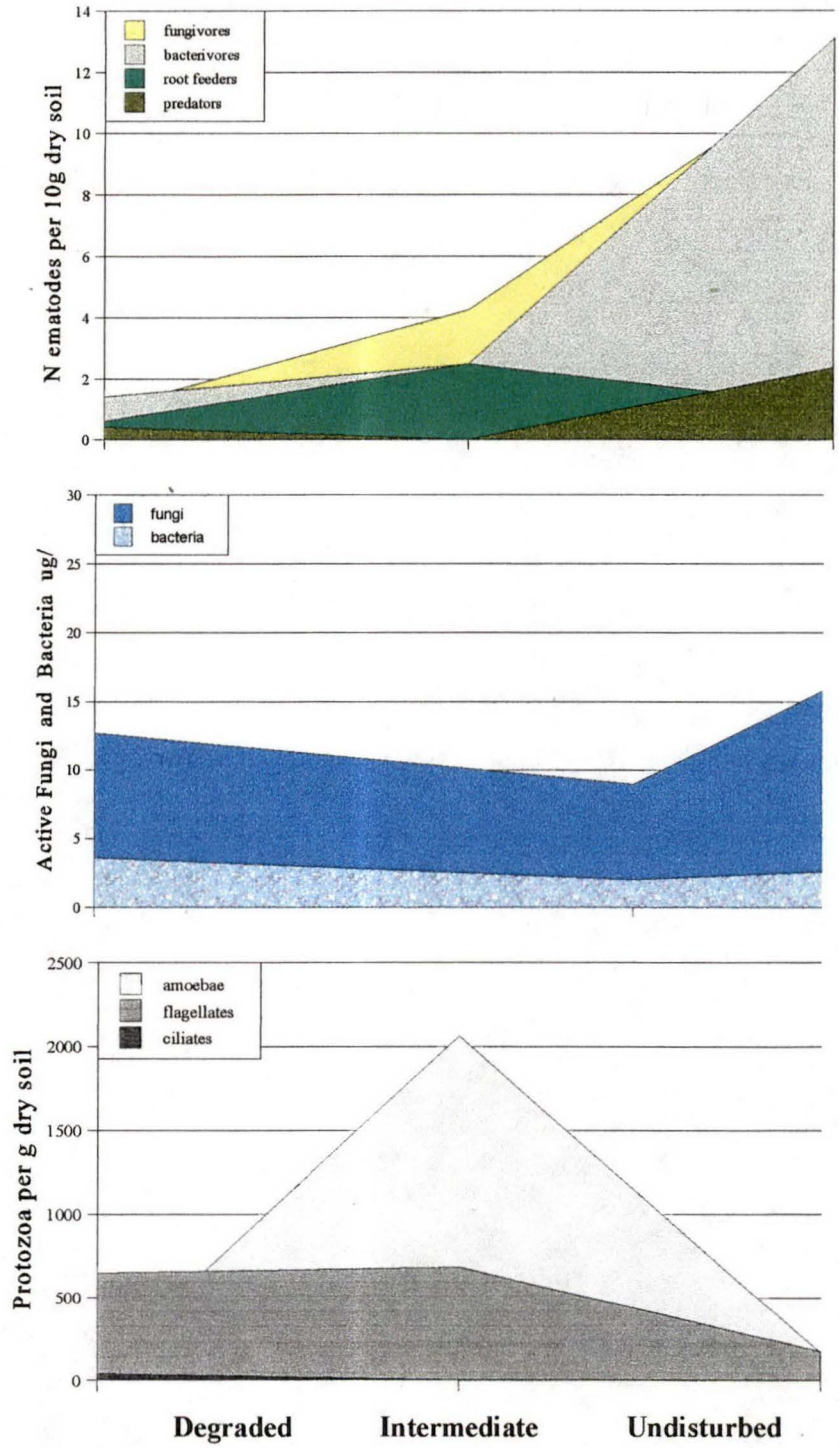

Figure 3. Relative abundance of soil organisms in the three soil types.

	Degraded	Deg *vs* Int p value	**Intermed.**	Int *vs* Und p value	**Undisturb.**	Und *vs* Deg p value
***u*g Active Bacteria per g soil**	**3.61** 1.6 n = 10	0.037	**2.02** 0.86 n = 10	0.015	**3.67** 1.22 n = 10	0.641
***u*g Active Fungi per g soil**	**12.71** 10.52 n = 10	0.492	**8.98** 7.57 n = 10	0.029	**28.42** 25.01 n = 10	0.116
Active Fun:Bac ratio	**3.78** 3.17 n = 10	0.508	**4.73** 3.53 n = 10	0.262	**7.4** 5.08 n = 10	0.083
***u*g Total Fungi per g soil**	**667.2** 517.8 n = 7	0.225	**933.9** 441.1 n = 7	0.063	**2155** 913 n = 3	0.010
Protozoa per g soil	**743.2** 465.1 n = 5	0.042	**2748** 2581 n = 5	0.001	**388.1** 229.2 n = 5	0.072
Nematodes per 10 g soil	**5.78** 2.9 n = 5	0.416	**13.62** 25.39 n = 4	0.013	**47.37** 23.41 n = 3	0.036
VAM Spores per g soil	**16.2** 13.04 n = 10	0.323	**22.4** 19.61 n = 10	0.776	**21.67** 18.99 n = 10	0.495

Table 3. Mean values for each organism class in boldface. Standard errors and sample sizes are listed beneath. P values are for Fisher's LSD pairwise comparisons of ranks.

Greenhouse Bioassay

Foliar N and P levels in the greenhouse-grown grass were significantly higher (1.7x and 1.3x, respectively) in plants grown in soils from the undisturbed site when compared to those in the degraded soils (Table 4 and Figure 4). Values for foliage from the intermediate soils were similar to those in the degraded soils. No mycorrhizal colonization was detected on the grass roots.

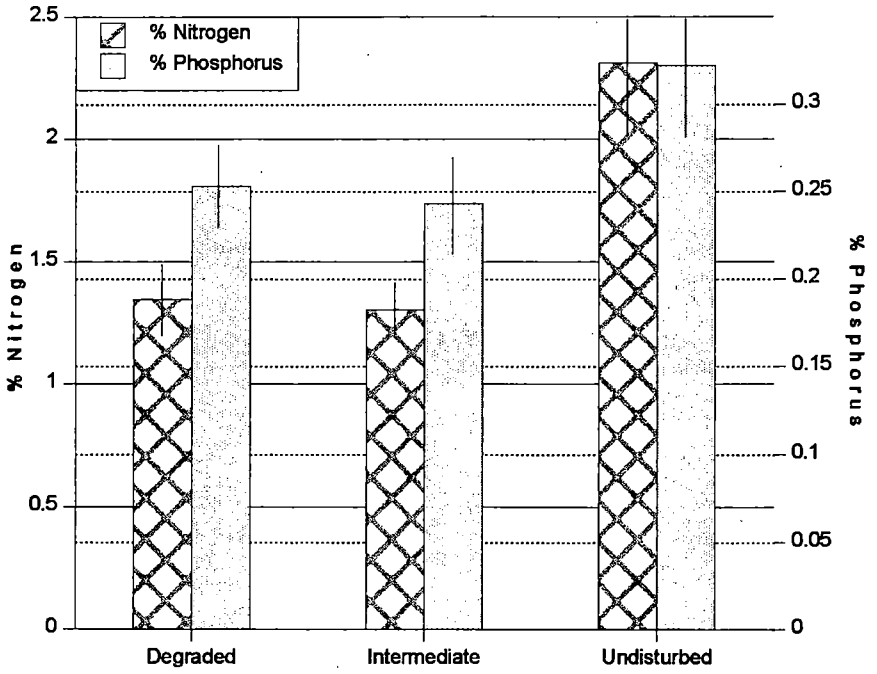

Figure 4. *Deschampsia* foliage N and P concentration. Error bars are standard error.

	Degraded	Deg *vs* Int p value	**Intermed.**	Int *vs* Und p value	**Undisturb.**	Und *vs* Deg p value
%N	1.344 0.145	0.762	1.302 0.111	≤ 0.001	2.311 0.273	≤ 0.001
%P	0.253 0.025	0.603	0.243 0.026	≤ 0.001	0.322 0.042	≤ 0.001

Table 4. Mean values for *Deschampsia* foliage N and P. Standard errors are listed beneath. P values are for Tukey pairwise comparisons.

Discussion

Foodweb

The analyses revealed disturbance-related shifts in both decomposer and consumer trophic levels of the soil foodweb. The activity of both bacteria and fungi was lowest in the area of intermediate disturbance, where vegetation was present but damaged and soil exposed between remaining plants was bare and compacted. The relative heterogeneity of the intermediate site could lend itself to a more diverse array of organisms, but it is unclear why the activity of decomposers would be significantly lower than in the degraded soil. Higher protozoan numbers in the intermediate site, attributable mostly to amoebae, suggest that all or a portion of that taxon may have been especially adapted to disturbance. High numbers of bactivorous protozoans could explain the decrease in their food base.

Taken together, the 10-fold decline in nematodes and the reduced supply of bioassay plant N and P in the degraded soils suggest discontinuity in the normal nutrient pathway from second trophic level output to plant uptake. That is, if bacteria and fungi are functioning as nutrients sinks and their consumers are absent, the nutrients tied up in decomposers will be unavailable to plants or the rate of supply will be diminished.

The degree of soil compaction associated with recreationists' trampling (exacerbated by vegetation loss) at Cobalt Lake may be such that soil pores have collapsed to a point where the mobility of meso- and macrofauna is restricted. Organisms belonging to the second trophic level typically require larger soil pore spaces than bacteria and fungi. Small pores (<2.5μm) associated with soil microaggregates are inhabited largely by bacteria, while large macroaggregates are richer in nutrients, with pores (25-100μm) more intensively colonized by larger organisms (Paul and Clark 1989). Hassink et. al. (1993) found close correlations between bacteria and pores <1.2μm and nematodes and pore sizes 30-90μm. They also found more N mineralized in soils where bactivorous activity was highest. Smeltzer et. al. (1986) reported significant declines in bacteria, fungi, nematodes and microarthropods following artificially induced forest soil compaction. Microarthropods (largely springtails and Collembola) that consume bacteria and fungi are the third major component of the

– releasing second level (Coleman et. al. 1990). Recent research in a subalpine forest in British Columbia revealed a sharp decline in fungivorous microarthropods and nematodes following logging disturbance (K. Johnson, unpublished).

Physical problems associated with forest soil compaction are well known (Greacon and Sands 1980; Childs et. al. 1989). Cole (1978) and Cole and Trull (1982) studied the experimental effects of controlled trampling on aboveground vegetation. Smelzer et al (1986) studied the effects of compaction on four classes of soil organisms, and Hassink et al (1993) studied the distribution of soil biota in soils of differing texture and pore size. We found no studies examining the effects of compaction on biologically mediated components of the belowground nutrient cycle.

Total Carbon and Nitrogen

Despite a significant decrease in total C in the degraded soil, 6.9% is a relatively high level of soil C, with regard to its physical and ion exchange site functions. The retention of water-holding capacity in the intermediate and degraded soils is probably due to the relatively high organic matter content. However, the remaining C stores may be in forms unavailable to the microflora causing them to be carbon (rather than nitrogen) limited. The decline in C:N ratio supports this. The absence of carbon compounds normally exuded by plant roots (Paul and Clark 1989) is likely to limit microbial populations that depend on rhizosphere health. The lack of significant difference in total N indicates that whatever changes occurred in nutrient cycle pathways as a result of degradation, including increases denitrification (see Section 2, this report), the overall soil N content has not declined.

Nitrogen and Phosphorus

Even though higher mineral N is frequently observed in disturbed soils (e.g. Khanna 1981; Vitousek 1981), the higher resin N values did not appear to accurately indicate higher availability to plants. In general, the resin results indicated higher mineral N in the degraded site, but bioassay plant uptake was significantly lower in the degraded soil. It is unclear whether the resin assay of the undisturbed site accurately reflected the same or less N

availability to roots during the assay periods, or failed to recover N as it cycled rapidly from pool to pool. N supply and limitation are often closely linked and differences between measurement methods stem from differences in the sensitivity of each method to factors influencing soil N transformations (Binkley and Hart 1989). Despite generally high levels of solution N present in the degraded soils, the rate of N turnover and supply to roots appears be higher in the undisturbed soils, as indicated by the bioassay results.

Because temperature, moisture and to some extent infiltration were standardized across all treatments in the greenhouse, the factors contributing to increased plant uptake may have been biological rather than physical. In the greenhouse bioassay the undisturbed soils likely had higher levels of both immobilizers (fungi) and mineralizes (nematodes) when they were collected for the experiment because they were subjected to the same collection and transportation conditions as those tested for the organisms. Whether they persisted throughout the growing period is unknown.

VAM colonization was absent from the bioassay plant roots, indicating that the increased uptake was not attributable to mycorrhizal function.

Phosphorus extracted from whole soil using dilute acid was also highest in the degraded site, again contradicting the foliar bioassay. Foliar P in the greenhouse bioassay was highest in the undisturbed soil. In the case of P however, resin measures did reflect plant availability: it was significantly higher in the undisturbed soil during the 5-week interval. There was no difference among the sites over the 10-month interval. It is unclear why resin sorption of P would be a more accurate index of plant availability than resin sorption of N. The difference may be related to differences in ion mobility: nitrogen ions are much more mobile in soil solution than phosphorus.

Specifically because plant bioassays are an index of nutrient availability integrated over time (Binkley and Hart 1989), thus incorporating supply rate, it appears that for this comparison between degraded and undegraded soils, the bioassay was the indicator of nutrient availability most relevant to restoration questions.

Conclusions

This study lends support to the idea that nutrient fluxes important to plants can be explained by the composition and function of soil organisms characterized as foodweb trophic levels. Soil foodweb structure and function is clearly far more complex than the simple relations tested here, but identifying critical disruptions in key nutrient pathways is a step toward ameliorating those disruptions with ecologically appropriate restoration techniques. In future research the questions raised here will be addressed by replicated studies using additional native plant species in paired field and greenhouse bioassays, combined with observations of the structure and function of the second trophic level. It will be necessary to determine whether the reduced nutrient availability or supply rate demonstrated in the greenhouse is a significant limitation on seedling establishment in field sites. Successful cultivation of native bioassay plants in degraded sites is quite labor intensive, and may require close attention to watering, shading, protection from damage etc, but in addition to testing the hypotheses presented here, the effort itself will lead to the development of further hypotheses about factors limiting site recovery.

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Increased bacterial denitrification in degraded soils

in two subalpine recreation sites

Summary

The conversion of NO_3 (nitrate) to N_2O gas (nitrous oxide) by soil-dwelling anaerobic bacteria, especially in disturbed sites, has received increased attention because it is well known as one of the "greenhouse gases" that may be contributing to global warming. Although the potential increase in N_2O efflux attributed to the two sites discussed here is undoubtedly orders of magnitude smaller than that from larger disturbances and naturally occurring anaerobic habitats, and perhaps not quantitatively important in relation to total site nitrogen pool, it indicates changes in belowground N pathways that may affect site recovery.

Potential dentrification was measured in degraded subalpine wilderness recreation sites as part of a larger study examining changes in soil foodweb structure and function. Surface soils were sampled in degraded, intermediate and adjacent plots at Cobalt and Red Eagle campgrounds in Glacier National Park. A laboratory measure of N_2O production was employed, under optimum anaerobic conditions for soil denitrifiers, holding glucose (food), moisture and temperature constant across all soil types. In samples from both campsites, N_2O production in the disturbed soils was roughly double that of the soils from the undisturbed areas. These results do not indicate N_2O efflux in the field, but indicate that denitrifier populations were significantly different in soils at the time of collection. That is, conditions in the degraded sites were more conducive to thriving denitrifier populations.

Decreased oxygen diffusion caused by compaction and increased nitrate (measured at Cobalt only) are two possible drivers of the observed increase. Soil temperature also limits denitrifiers and it is probable that surface soils in the open, more exposed degraded areas receive more sunlight and thus are warmer during the summer months.

Mean totals of active bacteria at the Cobalt site were not significantly different between the degraded and undisturbed areas, indicating a species shift occurred within the bacterial community in the degraded area without increasing overall numbers. At Red Eagle, however, overall bacterial activity was 2.5 times higher in the degraded area. It is not discernable from this data whether the increase in overall bacterial activity was attributable to denitrifiers. Other groups of bacteria also thrive in disturbed areas.

Both sites have been used as campsites for many years, yet at the Cobalt site there is

no difference in total soil N content, indicating that any overall loss of N in gaseous form has been minor, although the nitrate N pool that denitrifiers draw from is ephemeral and total N measures give do not indicate the relative composition of the soil N pools. In the degraded area at Red Eagle, however, where both denitrifier and total bacterial populations are higher, total soil N is 1.5 times lower than the undisturbed area. It is therefore possible that increased denitrifier activity has contributed, at least in part, to reduced soil N stores.

An increase in the movement of N from soil to atmospheric pool constitutes a change in N cycling pathways. If denitrifier populations are competing for nitrate that would otherwise be available to plants, or are contributing to a long-term decrease in soil N stores, then they may be affecting site recovery. The denitrifier activity measured in these sites may also serve as an "index" parameter that integrates a larger complex of related soil characteristics, particularly compaction (aeration), nitrate availability and increased soil temperatures.

Pinegrass (*Calamagrostis rubescens*) facilitates vesicular-arbuscular mycorrhizae colonization and phosphorus uptake in subalpine fir (*Abies lasiocarpa*; Pinaceae)

Abstract

An understanding of mechanisms of plant-plant facilitation and mycorrhizal symbioses among multiple plant species will be important tools in future restoration research and application. For this laboratory study we used two native montane plant species and vesicular-arbuscular mycorrhizae (VAM). An unusual aspect of this study is VAM colonization in a species of Pinaceae, a family previously regarded as ectomycorrhizal. Subalpine fir seedlings collected from high elevation sites have recently been reported to contain *Glomus*-type VAM root endophytes. The extent and ecological importance of the symbiosis is unknown. Specific objectives of this study were to examine a possible mechanism for interspecific, below-ground plant-plant facilitation by determining the role of a VAM plant host (a graminoid) on mycorrhizal colonization and nutrient uptake by subalpine fire. VAM spores were introduced into planting containers with fir growing alone and in combination with pinegrass. The same two treatments without spores served as controls. After 14 weeks fir roots were examined for VAM colonization and needles were analyzed for phosphorus and nitrogen concentrations. All inoculated fir seedlings grown in combination with pinegrass were colonized with abundant hyphae and vesicles. One inoculated fir seedling grown without grass formed two vesicles and a short length of hypha. No arbuscules were observed. Mean phosphorus content in the needles of colonized fir seedlings was significantly higher than in all other treatments. Phosphorus concentration in the colonized fir seedlings grown with the grass was 10.5 times higher than in the uncolonized fir grown with grass. Foliar nitrogen concentration did not differ between treatments. Foliar N:P ratios in colonized seedlings grown with grass averaged 7.5, while average N:P ranged from 55 to 167 in the other treatments. Optimum N:P ratios in other Pinaceae range from 3 to 7. This study provides evidence for a particular type of plant-plant facilitation involving species from two families via a generalist mycorrhizal fungus. The evidences suggests that the presence of VAM plant species could improve subalpine fir seedling establishment in the field, especially where ectomycorrhizal propagules are sparse or absent, and supports other studies showing that mycorrhizal fungi alter interactions between plant species.

Introduction

The Pinaceae have historically been regarded as an ectomycorrhizal group (Cázares and Trappe 1993; Read 1993; Gerdemann 1968), and published research on mycorrhizal function in that family is restricted to ectomycorrhizae. Recently Cázares and Trappe (1993) reviewed the few scattered reports of VAM (vesicular-arbuscular mycorrhizae) in Pinaceae and reported their own observations of frequent *Glomus*-type endophytes in seedlings of four species of Pinaceae collected in the wild, including subalpine fir (*Abies lasiocarpa*). Cázares and Smith observed VAM in two Pinaceae genera grown in a greenhouse soil bioassay (1992) and Smith et al. (1997, in review) successfully induced VAM colonization in seedlings with both spores and mycorrhizal companion plants. The ecological significance of VAM in the Pinaceae is unknown.

Because nutrient availability to plants is often restricted in the cold organic soils common to high elevations (Vogt et al 1989; Bowman et al. 1993), the role of mycorrhizae may be especially important. During periods of ecological stress VAM colonization may make a critical difference in seedling establishment and survival (Read 1991). Frequent observations of conifer sapling encroachment restricted to meadow edges led us to hypothesize that a scarcity of ectomycorrhizal roots and hyphae in meadows dominated by VAM and ericoid host plants limited successful establishment of subalpine fir seedlings at these high elevations. However, examination of cleared and stained fir seedling roots collected from the sites revealed vesicles, hyphae and occasional arbuscules typical of VAM, in addition to abundant ectomycorrhizae and unidentified dark-walled septate endophytes. Read et al. (1976) postulated that, in the absence of abundant spores, VAM plants establish by linking into existing networks of VA mycorrhizal hyphae. Based on evidence that interspecific plant hosts share common mycobionts and link below ground via fungal hyphae, Perry et al. (1989), Molina et al. (1992) and Read (1993) have argued for the existence of functional mycorrhizal guilds, wherein multiple species of plants benefit by hosting common fungi and sharing common mycelial networks.

Our initial observations of subalpine fir seedlings restricted to meadow edges

suggested that the ectomycorrhizal forest stands constituted functional guilds critical to fir seedling establishment under harsh environmental conditions. But the discovery of VAM colonization in young encroaching fir implied that membership in both VAM and ectomycorrhizal guilds might facilitate fir establishment and survival in marginal habitats, as well as migration into subalpine and alpine meadows, especially where a changing climate alleviates other factors affecting seedling establishment.

To determine in the greenhouse whether an interspecific VAM host was necessary for mycorrhizal formation in subalpine fir, and subsequent effects on fir nutrition we tested 1) whether *Glomus intraradices* would colonize subalpine fir from a spore inoculum, 2) the effect of an alternate VAM host on colonization and 3) effects of colonization on fir tissue N and P. The alternate host selected was pinegrass (*Calamagrostis rubescens*), which occurs commonly in forests and forest openings from valleys to subalpine zones in Pacific and Rocky Mountain forests (Lackschewitz 1991).

Materials and Methods

Treatments

Four treatments were applied in a 2X2 complete factorial design consisting of fir grown alone with no spore inoculum (F), fir grown together with grass (F+G), fir grown alone with spores (F+S) and fir grown with both pinegrass and spores (F+G+S). Each treatment was replicated 49 times in a completely randomized fashion and mortality reduced replications to 41, 41, 37 and 34, respectively.

Seed germination and growing medium

Subalpine fir seeds were surface-sterilized in 30% hydrogen peroxide for 30 minutes, then cold/moist stratified in vermiculite for 10 days at 4C. Seeds germinated at room temperature and one 10 day old seedling was transplanted into each 150cc Supercell™ leach tube (Stuewe & Sons, Corvallis, OR). Seeds of pinegrass were treated briefly with a Captan™ fungicide solution (Zeneca, Wilmington, DE) and planted in the leach tubes at the same time

the fir seedlings were transplanted. The growing medium consisted of 4 parts horticultural grade vermiculite and 1 part horticultural grade heat-expanded pumice.

Inoculation

The spore Nutrilink™ inoculum (Native Plants Inc., Salt Lake City, UT) in a dried clay carrier contained approximately 500 spores of *Glomus intraradices* per gram. Uninoculated controls were treated with an equal amount of dried clay carrier containing no spores. The growing medium was moistened prior to planting and a narrow glass rod was then inserted in the center of the mix to create an open column for inserting 0.4mls of inoculum followed by the fir germinant. Pinegrass seeds were scattered on the surface and covered lightly with growing mix.

Growing conditions

The plants were grown in a laboratory at 20-22C under 16 daily hours of artificial Gro Lux™ light (Sylvania, Hillsboro NH). Seedlings were watered as needed to maintain media moisture and fertilized four times during the course of the experiment with Long-Ashton nutrient solution (Hewitt 1966) modified to reduce phosphorus concentration to one-quarter strength (11ppm). Each seedling received a total of 100 mls of nutrient solution over the course of the experiment.

Clearing and staining

Seedlings were harvested after 14 weeks. The clearing and staining procedures are modified from Cázares and Trappe (1993) and Phillips and Hayman (1970). Whole root systems were washed in running tap water, placed in Tissue-Tek™ plastic capsules (Fisher Scientific Co., Pittsburgh, Pa.) and soaked in 10% KOH solution for 14 hours. The KOH solution was decanted, samples rinsed with distilled water and soaked again in 10% KOH for 7 hours. Roots were then rinsed again with distilled water, placed in a fresh 10% KOH solution, steamed for 40 minutes, rinsed, steamed for 30 minutes in 1% HCL and rinsed again. Cleared samples were steamed for 30 minutes in a solution of 0.05% trypan blue in

lactoglycerol, rinsed and stored in lactoglycerol until microscopic examination. A small subsample of grass roots was also cleared and stained using the same methods for comparison between inoculated and uninoculated treatments.

Leaf tissue analysis

All needles were removed from ten randomly selected seedlings in each of the four treatment groups and dried overnight at 70C. Tissue analysis for percent nitrogen and phosphorus used a micro-Kjeldahl digest method following Bremner and Mulvaney (1982). Several of the samples in the uncolonized treatments had tissue P levels below the 0.01% detection limit in the Kjeldahl test. In order to calculate N/P ratios, a value of 0.01 was assigned to those samples, so ratio estimates for those treatments represent minimum values. Included in the analysis were needles from 3 wild subalpine fir seedlings to provide a basis for nutrient level comparisons.

Assessing colonization

For all fir seedlings each 5mm section of the entire root system was systematically examined from root collar to root tip with a Zeiss binocular microscope for the presence of vesicles, arbuscules or hyphae. Root systems were not heavily lignified so evidence of fungal activity was easily discerned. If any portion of a 5mm segment contained evidence of VAM it was tallied as colonized. Each entire root system was counted as one sample. Percent colonization quantities given below represent percent of segments in a root occupied by at least one of the forms of the fungus. This estimate of colonization does not provide a complete estimate of percent root length colonized because not all segments were entirely colonized, but does provide a measure of the distribution of colonization throughout the root system (Read et al. 1976).

Statistical analysis

Analyses of foliar nutrients for each treatment were conducted on randomly selected samples of 10 using the Multifactor ANOVA procedure and Tukey tests for multiple

comparisons in Statgraphics™ (Statistical Graphics Corporation, Rockville MD). Phosphorus data were square root transformed to stabilize variance, using the equation $X' = X + X+1$ (Zar 1984).

Results

No fungal colonization was detected in fir roots from the F or F+G treatments. No colonization was detected in a subsample of pinegrass roots from the uninoculated treatment. One of the fir root samples from the F+S treatment contained two vesicles and a 1mm length of hypha. All root samples from the F+G+S treatment contained vesicles and/or hyphae, usually both. Colonization was abundant: 97% of the root samples were $\geq$ 25% colonized; 50% of samples were $\geq$ 50% colonized; 15% of samples were $\geq$ 75% colonized. Colonization was not evenly distributed between main and lateral roots. Sixty-five percent of lateral roots had less than 25% colonization. Only 21% of lateral roots were $\geq$ 50% colonized, compared to 79% in main roots. Vesicles and hyphae were infrequent in root tips. No arbuscules were observed in any samples. A subsample of pinegrass roots from this treatment showed typical VAM colonization. Visual assessment at harvest time revealed no obvious differences in seedling size or vigor between treatments.

Nitrogen concentrations in the experimental seedlings did not differ significantly among treatments. Seedlings from the F+G+S treatment had significantly higher P concentrations than the other three treatments ($p<0.01$), and a highly significant interaction occurred in the F+G+S treatment ($p<.001$). Differences among the other three treatments were not significant at a 0.05% level. Foliar N and P concentrations and N:P ratios are summarized in Table 1. N and P concentrations in the 3 field specimens were consistently lower than the experimental seedlings, but the N:P ratio was similar to that for the F+G+S treatment.

	Fir **n = 10**	**Fir + Grass** **n = 10**	**Fir + Spores** **n = 10**	**Fir + Spores + Grass** **n = 10**	**Field Samples** **n = 3**
% Nitrogen	**2.88**a 0.14	**2.49**a 0.09	**2.61**a 0.17	**2.48**a 0.18	**0.11** 0.02
%Phosphorus	**0.08**a 0.02	**0.04**a 0.02	**0.14**a 0.05	**0.40**b 0.06	**0.01** 0.003
N:P Ratio	**90.48** 30.61	**167.35** 35.84	**55.08** 26.75	**7.51** 1.03	**8.80** 0.66

Table 1. Mean percentages (in boldface, not transformed) of foliar nitrogen and phosphorus, and percentage ratios. Standard errors are listed below each mean. Values followed by different letters are significantly different at $p \leq .05$ (from Tukey's pairwise comparisons on log-10 transformed data).

Discussion

The general absence of colonization in the F+S treatment suggests that colonization of fir roots in the F+G+S treatment occurred via hyphae growing from the roots of colonized pinegrass. This conclusion is supported by evidence for interspecific hyphal colonization by VAM (Francis et al. 1986) and evidence that not spores, but fungal hyphae associated with established plants, are the major source of VAM inoculation in natural communities (Read et al. 1976; Read 1993). Spore abundance in natural communities can be low despite abundant colonization of most plants (Read 1976).

Because *Glomus* spore germination may be stimulated by root exudates of some plants (Graham 1982), it is possible that spore germination was stimulated by a grass exudate of different quantity or quality than the fir, leading to subsequent colonization of fir roots by spores. The presence of hyphae and vesicles in one fir from the F+S treatment indicates that at least one spore germinated in the absence of grass.

Comerford and Fisher (1984) determined that a foliar N:P ratio greater than 14 or 15

indicates a sufficiency of N with respect to P while a lower ratio indicates that N is limiting. The high N:P ratios in the three uncolonized treatments indicate that P may be severely limiting in those seedlings. Comparisons with foliar N:P ratios from the wild seedlings and ratios derived from other conifer studies (Ingestad 1979; Comerford and Fisher 1984; Munson and Bernier 1993) suggest that the N:P ratio in fir under the F+G+S treatment is probably within the normal and perhaps optimum range for growth. Because the experiments were not conducted in sterile microcosms we have not proven a direct causal link between *Glomus* colonization and increased tissue P. However it is widely accepted that VAM improve host P nutrition. Other VA mycorrhizosphere organisms also influence plant nutrient uptake (Barea et al. 1975; Hayman 1982), and their role, if any, should be determined.

VAM colonization did not alter foliar N concentration in the test seedlings, but the full strength N fertilizer resulted in foliar N concentrations considerably higher than the three wild seedlings and values reported for other conifers (Ingestad 1979; Binkley 1986; Munson and Bernier 1993). Slow growing plant species adapted to infertile sites may absorb nutrients in excess of immediate growth requirements (luxury consumption) to support growth during later periods of nutrient stress (Chapin 1980). Such an adaptation in subalpine fir could explain the high amounts of foliar N measured in this study.

Our experimental evidence of shared compatibility between plants of different families for a common mycorrhizal fungus demonstrates the potential for linkage between those plants in the wild, and suggests that the composition of mycorrhizal guilds can extend between VAM and ectomycorrhizal plants. The findings support the hypothesis by Read et al. (1976) that VAM plants establish and survive by linking into existing hyphal networks. The potential complexity of guilds that integrate multiple species of plants and fungi through space and time (Molina et al. 1992), belies a simplistic view of interconnected plants. Observations by Cázares and Trappe (1993) that VAM colonization levels were lowest in *Abies* seedlings collected under a closed forest canopy led them to hypothesize that the higher levels of colonization in seedlings collected from openings was related to a greater abundance of VAM hosts and propagules; implying also that the VAM hyphal network is sparser under the forest canopy. Preliminary results of a soil transfer experiment near treeline in Montana (K.

Johnson, unpublished) show that after 2 growing seasons ectomycorrhizae-bearing forest soils transferred to meadow and alpine planting sites improve the survival and growth of transplanted lodgepole pine germinants, but not that of subalpine fir. The lodgepole pine may be more dependant on ectomycorrhizae for seedling establishment.

According to Francis et al. (1986) the increase in tissue nutrients derived from the mycorrhizal symbiosis may be more important for survival of establishing seedlings than increases in growth. Further study should test the conditions under which VAM-induced alleviation of P deficiency improves the chances for seedling establishment and survival. Because VAM also improve plant access to water and other nutrients (Allen 1991), the symbiosis has the potential to facilitate fir establishment where these resources are limiting, if the benefits of hosting the fungi outweigh the costs.

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Effects of three *Phialocephala*-like fungal inocula on survival and nutrient status in *Abies lasiocarpa* and *Pinus contorta*

Summary

There is little information available on high-elevation mycorrhizal ecology, and few experimental studies have been published. The research described here focuses on a specific class of fungi, not always regarded as mycorrhizal, that is quite common in subalpine and alpine habitats, and provides evidence for its potential use as a restoration tool. The fungi are characterized in the literature as "dark-septate" (on the basis of hyphal color and morpholgy), or *"Phialocephala*-like" because of their similarity to a known genus. OSU researchers sampling 52 subalpine plant species detected root colonization by these fungi in 71% of the species. They occurred in all major life forms, i.e. trees, shrubs, grasses and forbs, spanning 14 of the 16 families sampled. Neither ecto- nor vesicular-arbuscular mycorrhizae are as diverse at this level. Most of the dark-septate isolates have proven difficult or impossible to place taxonomically. The fungi are root endophytes but do not form typical mycorrhizal structures. Recent studies suggest, however, that at least a subset of the group may form mutualistic symbioses with host plants. On the basis of those findings we chose to conduct experiments to determine 1) if quantities of inoculum for greenhouse and field studies could be easily propagated in the laboratory, 2) what effect the fungus has on a plant host in the presence and absence of organic matter, and 3) differences in effects of 3 different isolates on two conifer species. Preliminary findings of an ongoing field study are also mentioned.

Efforts to isolate and propagate numerous cultures were successful. If handled using standard sterile techniques, the isolates grow readily at room temperature in liquid media mixed with vermiculite.

The first plant growth experiment was conducted in a laboratory environment, under artificial light, using *Abies lasiocarpa* and an isolate from a heather species. Fir germinants were transplanted into soil-less growing media consisting of vermiculite and perlite, plus 1) fungal inoculum, or 2) peat moss or 3) both fungal inoculum and peat moss. After 13 weeks seedlings were harvested, inspected for root colonization and foliage analyzed for N and P content. Roots from the two treatments containing fungal inoculum showed evidence of sparse colonization typical of dark-septate endophyte morphology. Mean foliar P in the treatment containing peat and fungus together was double that in the other two treatments

($p \leq .001$). Foliar N did not follow this pattern. The treatment with peat alone led to significantly higher N ($p \leq .001$) than the other two treatments. The results suggest that the organic substrate provided by peat interacted with the fungus to increase tissue P, although the exact mechanism is not known. Because foliar N was higher in the treatments without it, the fungus appears to have acted as an N sink . Despite this, the ratio of N to P in all treatments was at or above normal (ranging from 7 to 34) indicating that fungal N sequestration did not lead to nutrient deficiency.

A second experiment, conducted in a greenhouse with supplemental light, tested the effects of 3 fungal isolates and an uninoculated control treatment on *Abies lasiocarpa* and *Pinus contorta*. An isolate of the original *Phialocephala fortinii* type culture, the heather isolate used above, and an isolate from *Pinus albicaulus* were used. The growing medium consisted of pasteurized subalpine soil amended with perlite. As above, the isolates were introduced in a vermiculite carrier. Seedlings were harvested after 8 months. No colonization typical of dark-septate endophytes was observed. All seedlings, including those in the control treatment were heavily colonized with two ectomycorrhizal species considered greenhouse contaminants. However, despite the apparent absence of root colonization by dark-septates, foliar N and P in *Pinus* were significantly higher in the treatments containing dark-septate isolates ($p \leq .001$). No treatment differences were detected for *Abies*. It is possible that the roots were colonized early in the experiment but were no longer colonized at the time of harvest, or that colonization was below detection levels. It is also possible that the fungal inoculum introduced in the vermiculite carrier provided a compost-like organic substrate conducive to a flourishing microbial community that became beneficial to plant nutrient uptake. Reports in the literature of mycelium-dwelling microbial communities, including nitrogen fixers and other beneficial organisms, support this speculation, and it is testable, as is the question of whether it was necessary for the fungal substrate to be living.

Evidence from these experiments suggests that not only are *the Phialocephala*-like endophytes not pathogenic, as reported elsewhere, but they may be beneficial as mutualistic colonizers of plant roots, or perhaps act alone as a beneficial soil amendment under certain conditions. As discussed in Section 5, preliminary results of a field experiment at Cobalt Lake using inoculated sedge plants show 100% survival in an inoculated group, compared with 61% survival in an uninoculated group. Concurrent work by other researchers at OSU indicates that these fungi produce decomposition enzymes that may allow them to directly access major nutrients tied up in soil detritus.

That the fungus alone might act to amend dead or degraded soils is a potentially

important aspect of these results. Laboratory and field studies addressing this question are warranted. As mentioned above, this class of fungi is widespread and occurs in many plant species in high-elevation systems. Despite taxonomic problems that have hampered basic study of this group, they are relatively easy to isolate and grow, and would lend themselves well to large scale revegetation experiments with multiple plant species. In situations where the genetic integrity of native communities is a special concern, as is the case in national parks, it should be particularly easy (and uninvasive) to obtain viable isolates near restoration sites.

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Summary of Seeding Experiment

Beargrass (*Xerophyllum tenax*) and Hayden's sedge (*Carex haydeniana*) were selected for this experiment based on their abundance and wide distribution, tendency to resist trampling, and information about germination requirements. Work by this author and others in the Glacier Park native plant nursery has shown that good germination of these two species can be obtained by sowing seeds in containers in the fall and overwintering them outdoors, thus meeting the requirement for a very long, cold, moist stratification. Efforts to stratify both species indoors for several months in moist sand at 4 degrees C have not been successful. In this experiment we attempted to meet stratification requirements in the field, while protecting seeds from wind and predation.

In October 1994 replicate seed plots were established in two degraded areas at Cobalt Lake. Plots were 4m square, each containing 40 subplots randomly located on grid intersections. Subplots were 15cm square. Locations of subplots were recorded by determining their "coordinates" relative to two plot edges serving as X and Y axes.
All subplots were seeded with a mixture containing approximately 160 seeds each of beargrass (*Xerophyllum tenax*) and Hayden's sedge (*Carex haydeniana*). Seeds were not covered with soil, in order to mimic natural seedfall. One third of the subplots were covered with $15cm^2$ fine mesh steel screens held in place with small nails. In June, immediately following snowmelt, screens were removed and all subplots were examined for seeds. All screened plots still contained seeds. No seeds were located in any of the other subplots. At the end of the growing season subplots were examined again and no seeds or germinants were found.

Summer rodent activity appears to be especially high at Cobalt Lake, probably because of humans and their food, so seed predation may have been particularly intense. In the future, over-winter protection, combined with spring and summer germinant protection may allow for germinant survival. Treatments that combine raking seeds into the surface soil then protecting with screens might be more effective. Because the protective screens in this experiment were placed flat against the ground, retaining them in the spring would not have

allowed for germination. A second set of replacement screens placed following snowmelt, but raised a few centimeters to allow for germination, might sucessfully protect young seedlings. We note that earlier seeding trials in another area of the Park, using raised screens installed in the fall, failed because screens were crushed to the ground by snow. Though labor intensive and unsightly, the two-step process of screening may be the only effective way of protecting seeds sown in the fall.

Summary of *Carex* Field-Plot Results

This experiment was designed to test the effects of perlite added to planting soil for the purpose of improving root zone aeration, and the effects of a potentially mycorrhizal *Phialocepha-like* fungal inoculum (described in Section 4). An untreated control was included. *Carex haydeniana* transplants were 2 yr-old, robust, well-fertilized, nonmycorrhizal nursery stock from the Glacier Park native plant nursery. Eight weeks prior to outplanting all plants were root- and top-pruned and replanted in flats of moist vermiculite. For the inoculation treatment roots were exposed to fungal inoculum propagated in vermiculite. Transplants treated with perlite were planted with 200cc of perlite introduced into planting holes at the time of planting. Controls were moved from the vermiculite flats to planting holes without treatment. All plants were watered with lake water during transplanting and three subsequent times at one-week intervals.

Results listed here suggest that the fungal inoculation was beneficial to survival, but not necessarily to first-year seed production. The perlite treatment appears to have improved survival in plot 5, which is the wetter of the two sites. Flowering was highest in the perlite treatments. Plots will be monitored again during the 1997 growing season.

	%SURVIVAL		% OF SURVIVORS PRODUCING SEED	
	Plot 4	Plot 5	Plot 4	Plot 5
Control	12/13 = **92**	8/13 = **61**	8/12 = **67**	4/8 = **50**
Perlite	12/13 = **92**	10/13 = **77**	10/12 = **83**	9/10 = **90**
Fungus	14/14 = **100**	14/14 = **100**	9/14 = **64**	12/15 = **80**
Plot Total	38/40 = **95**	33/41 = **80**	27/38 = **71**	25/33 = **76**

First year results of *Carex* plots. Percent survival and seed producers are in boldface.

Conclusions and Recommendations

The findings of the Cobalt Lake case study present a strong line of evidence for belowground biological degradation occurring concomitantly with soil compaction and vegetation loss at the campsite. They are a first step in understanding how the living plant-soil system unravels following disturbance as seemingly uninvasive as human footsteps. The most significant findings of the baseline analyses described in Section 1 are that the recreational activities led to a decline in soil organisms that normally perform critical functions in the cycling of major plant nutrients, and that native grass grown in the degraded soil contained significantly less nitrogen and phosphorus. The significant decline in soil organisms may actually be underestimated in this study because large numbers of organisms adhere closely to live roots and mycorrhizal hyphae and may not have been fully accounted for by our methods.

Even though bacterial activity appears unchanged across the disturbance gradient, the results of the denitrification analyses show that the composition of the bacterial community has changed, affecting the nitrogen pathway. Abnormally high populations of denitrifiers, which rely on nitrate for energy, would divert it away from plants. It is noteworthy that in agricultural studies the addition of nitrate fertilizer sometimes exacerbates the problem by feeding the denitrifiers, rather than the plants.

The relationship between the decrease in nutrient uptake by the bioassay plants and the shifts in soil foodweb should be tested further and specific mechanisms examined closely, because a depressed nutrient supply rate could contribute to germinant or transplant failure, especially in harsh environments. Experiments could be designed that monitor the soil foodweb in field and greenhouse bioassays, isolating the effects of decomposer and consumer groups, including microarthropods. Techniques for speeding microbial and microfaunal recolonization of degraded areas should be explored (see Curry and Good, 1992, cited in Section 1). We propose that nematodes, and perhaps microarthropods be used as index groups where soil compaction is a disturbance factor, and that future research examine relationships between soil structure and consumer group activity. Coupled with a native plant bioassay, that index could go further toward identifying a potentially critical nutrient

cycle disruption.

Also important is the greenhouse finding that one plant species can indirectly improve the nutritional status of a second species via the facilitation of mycorrhizal colonization. That the second species was a subalpine conifer widely believed to be ectomycorrhizal is an unusual finding, and contributes to our knowledge of its autecology. It also contributes to the work of other OSU researchers who have detected VAM colonization in field-grown seedlings of subalpine fir. Evidence in the literature supports the idea of mycorrhizal "companion" plants and mycorrhiza-mediated plant interactions.

The lack of VAM colonization in the bioassay grass was surprising, because it is probable that *Deschampsia* normally hosts VAM fungi. Evidence in the literature (and results of the fir/pinegrass experiment) suggests that VAM hyphal fragments lose viability quickly and serve best as inocula when they are part of a living, intact mycelial network. Digging and transporting samples to the greenhouse undoubtedly reduced the VAM inoculation potential of to the soil. Together the experiments demonstrate the importance of an intact, living network of roots and hyphae and highlight the significance of neighboring plants as facilitators, rather than competitors.

The results of the two greenhouse and ongoing field experiments with the dark-septate, *Phialocephala*-like fungi confirm their potential as a restoration tool. As described in Section 4, they colonize many subalpine and alpine plants and are relatively easy to isolate and propagate. Conclusions from this and other studies are mixed, but some show that members of this class of fungi have the ability to improve plant nutrient status and biomass following root colonization. No research has been conducted to test whether these fungi mediate intraspecific plant interactions. The results of the second greenhouse experiment in Section 4, where all three isolates of the fungi failed to colonize roots, but nevertheless improved seedling nutrient status, indicate that the living fungal mycelium should be tested as a "native" beneficial soil amendment in revegetation experiments. At least one type of fungal material is already available commercially as an organic soil amendment, irrespective of its non-symbiotic role (Biosol™, Rocky Mountain Bio-Products, Inc., Edwards, CO.).

Future research and experimental management efforts can draw from these findings through the development of carefully timed, stepwise treatments targeting specific classes

of organisms. Direct seeding techniques should be tested, where germination requirements are known. Experiments could be devised that incorporate over-winter seed protection coupled with spring germinant protection. The timing of the introduction of fungal inoculum, depending on its viability, could be varied, as could the introduction of whole-soil transfers from nearby areas. Previous experiments have shown that only small amounts of soil are needed for this technique, but timing may be important. The same arguments hold for nursery-grown transplants - whether inoculum should be introduced in the nursery or the field will probably differ for different species of plants and fungi. Preliminary results of the *Carex* field plots suggest that greenhouse inoculation with dark-septate fungi may be effective.

On the basis of our findings and those of others, we suggest that native grasses deserve more attention as a high-elevation restoration tool. They grow fast, are not typically difficult to germinate, and are an abundant annual source of leaf litter. They allocate more energy and resources to root production than many other species. Root production and turnover is critical to numerous physical, chemical and biological properties in healthy soils. Rhizomatous grasses often spread quickly and might be more resilient to foot traffic than fragile herbs or slow-growing graminoid or woody species. Grasses are routinely used in mycorrhizal research to provide a source of VAM inoculum. Roots from these "pot cultures" containing vesicle and hyphae are harvested, chopped, and mixed into the growing medium of other experimental plants. In many years natural seed production is low at high elevations, but limited research by others (e.g. In the Glacier Park native plant nursery) has shown that subalpine and alpine plants, including grasses, can be propagated for seed production at lower elevations. We encourage more work in this area. Finally, the results of the fir/pinegrass experiment reported here demonstrate the potential of grasses as companion plants, and the greenhouse biossay with *Descahampsia* indicated its usefulness in as an integrated index of nutrient limitation in degraded soil.

The relative complexity of native systems belies hope that broad or simple methods to facilitate plant and soil productivity, like those used in agriculture and horticulture, will ever be widely applicable. Each place will have unique above- and belowground species assemblages and environmental characteristics (including disturbance intensity), that necessitate a site-specific synthesis of choices about amendments, nutrient additions and

species selection.

A reasonable hope might be to develop principles that are applicable within the constraints of a given habitat, for example cold subalpine, where soils are thin, temperature limited, highly organic, and act as a nitrogen sink. Then, within that habitat, it would be necessary to determine whether the plant community hosts predominantly VAM, ectomycorrhizal, ericoid mycorrhizal or dark-septate fungi. Different choices would be made depending on whether the goal is to restore the site to its original array of diversity or protect soil and prevent further damage where impact is likely to continue. For now, at least, the quality of synthesis required, and its dependence on detailed knowledge of local communities pushes the discipline of ecological restoration toward the intersection of science and art.

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